THE SECRETORY CAPACITY OF THE STOMACH OF THE WOMBAT (VOMBATUS HIRSUTUS) AND THE CARDIOGASTRIC GLAND

G. W. Milton,* D. J. Hingson† and E. P. George‡

(*Communicated by Dr. Mervyn Griffiths*)

[Read 27th March, 1968]

Synopsis

The secretory capacity of the stomach of the wombat (*Vombatus hirsutus*), and of the cardiogastric gland of this animal has been studied.

It was found that the secretory power of this stomach resembles that of man and animals commonly used in gastric research. The concentrations of ions in gastric juice generally fitted the Hollander two-component theory of gastric secretion. A close correlation between the concentration of pepsin and of K^+ was demonstrated. The electrophoretic pattern of the gastric juice of the wombat resembled that obtained from the gastric juice of man. The maximum secretory capacity of the stomach of this animal was lower than that of man. It was found that a considerable increase in the gastric output could be obtained by augmenting the effects of histamine stimulation by injections of insulin.

Introduction

In three mammals, the wombat (*Vombatus hirsutus*), the koala "bear" (*Phascolarctos cinereus*) and the North American beaver (*Castor canadensis*) there exists on the lesser curvature of the stomach a highly specialised area of secreting cells, known as the cardiogastric gland (Home, 1808; Johnstone, 1898; Mackenzie, 1918; Milton, 1962). The anatomy of this gland has been studied in the beaver (Smith *et al.*, 1911; Nasset, 1953) and in detail in the wombat (Hingson and Milton, 1967). In the latter animal the gland consists of about 20 infoldings of the gastric epithelium with the muscularis mucosae and submucosa. Each of the gland pits opens into the lumen of the stomach through a separate ostium. The pits are lined with thickened gastric epithelium which contains innumerable gastric glands. The cells in these gastric glands are of the usual type found in the lining of a carnivorous mammalian stomach. The chief and parietal cells are particularly prolific and the gland tubules appear to possess a good blood supply from the left gastric artery. A separate branch of the vagus nerve enters the gland from the parietal surface. The remainder of the stomach in the wombat has a lining similar to that of other mammals, the antrum is well defined and consists of non-acid secreting epithelium. There is also a small band of non-acid secreting epithelium close to the oesophageal opening. The body of the stomach is lined with glands similar to other animals. The glands here consist of surface mucus cells, mucus cells at the neck of the gland, chief and parietal cells and an occasional argentaffin cell.

Each of the animals possessing a cardiogastric gland is a herbivore. The precise diet of the wombat is not fully known, but it probably consists of roots and grasses that are found near its burrow. Both the wombat and the

---

* Department of Surgery, University of Sydney, Sydney.

† Research Student, Johns Hopkins University, Baltimore, U.S.A.

‡ School of Physics, University of New South Wales, Sydney.

koala are large marsupial animals and are therefore widely separated in evolution from contemporary mammals such as the beaver.

The object of the present paper is to report a study of the secretory function of the wombat stomach and of the cardiogastric gland. We have also investigated the ionic relationships in the gastric juice of this animal to ascertain if they are in agreement with the Hollander two-component theory of gastric secretion (Hollander, 1932).

MATERIAL AND METHODS

Nine adult healthy wombats weighing between 13–25 kg. were used in these experiments. The animals were fasted for 18–24 hours and anaesthetised with nitrous oxide and oxygen mixture administered through a face mask. As soon as anaesthesia had been induced a fine intravenous catheter was inserted into one femoral vein and passed into the inferior vena cava. The catheter was attached to a three-way tap and through it a connection made to a syringe containing dilute pentothal sodium (1 gm./60 ml.). Anaesthesia was maintained by occasional injection of 1–3 ml. of this solution. Throughout all experiments the animal was given a continuous infusion of histamine acid phosphate (2·2 μg. base/kg. body wt./min.) administered by a slow infusion pump. In several experiments once a steady baseline of secretion had been established booster doses of histamine were administered (0·5 mgm./kg.) subcutaneously. After some hours of histamine the animals were given a subcutaneous injection of soluble insulin (50 units).

The experiments were divided into two groups. In Group 1 the secretion from the whole stomach excluding the antrum was studied. An intragastric tube was passed through the mouth and its position checked by opening the abdomen. A tight ligature was then passed around the antral region just distal to the end of the tube, thereby excluding the antral secretion from the rest of the stomach. In Group 2 the stomach was opened along the anterior surface and a suction tube passed through the mouth and fixed over the openings of the cardiogastric gland by sewing the tip of the tube to the lesser curve just beyond the gland. The gland area was then excluded from the remainder of the stomach by sewing together the anterior and posterior gastric surfaces across the tube with an atraumatic stitch. In this way a pouch was made which consisted largely of the cardiogastric gland. A second tube was placed in the remainder of the stomach through the gastrotomy opening and brought out through the anterior abdominal wall. The tubes used to aspirate the gland area and the stomach were double lumened, the smaller lumen being an air inflow to prevent excessive suction. When the tubes were in place the caudal end of the table was raised eight inches to prevent the saliva from trickling into the stomach and to facilitate aspiration.

At the conclusion of the experiment the position of the tubes was checked and also the patency of the septum between the cardiogastric gland and the body of the stomach.

The samples of gastric juice were collected under ice and the collecting cylinder changed half-hourly. After insulin injections the collecting cylinder had to be changed more frequently (10–15 minutes). Each specimen was tested for the following: (1) Acidity by titration against NaOH (N/50) to pH 7·4, using a glass electrode pH meter coupled to an automatic titrator (Radiometer). (2) Na^+ and K^+ by flame photometry (Perkin Elmer, external standards). (3) Cl^- by potentiometric titration using the automatic titrator (Lehmann, 1939; Muller, 1942). (4) Pepsin using the haemoglobin substrate method (Harrison, 1964). (5) Total osmolarity was measured in seven samples of high concentration using a freezing point osmometer. (6) In one

animal the stomach was irrigated with $NaHCO_3$ by the technique of Piper *et al.* (1963) and the electrophoretic pattern of the gastric juice estimated by Piper (1966).

Samples of blood were taken from five animals and the total osmolarity, Na^+, K^+, and Cl^- concentration of the plasma were measured by the above techniques.

The figures were analysed in several ways. The interrelationships of ions, volume, pepsin for both concentration and output were determined with the assistance of the digital computer, SILLIAC. The correlation coefficients between pepsin and H^+, K^+, and Cl^- were obtained. In addition observations were made on the response to histamine, and histamine augmented with subcutaneous histamine and subcutaneous insulin.

RESULTS

The characteristics of the serum of the wombat are shown in Table 1, together with the total osmolarity of gastric juice secreted at maximal acid concentration. It can be seen that the osmolarity and concentration of ions in the serum of these animals is roughly the same as that in other animals more frequently used in gastric research. The osmolarity of human, dog, and cat plasma ranges from 300 to 330 m. osmoles/litre (Houssay, 1955; Spector, 1956).

TABLE 1

Serum osmolarity and the osmolarity of concentrated gastric juice in milli osmoles/litre, and the concentration of Na^+, K^+ and Cl^- (mEq/l.) from the serum of the wombat, $\pm S.D.$ The figure in brackets is the number of specimens analysed

Total osmolarity serum	$286 \cdot 2 \pm 20 \cdot 8$ (5)
Ions (serum) :	
Na	$134 \cdot 8 \pm 3 \cdot 3$ (5)
K	$4 \cdot 4 \pm 0 \cdot 35$ (5)
Cl	$96 \cdot 8 \pm 10 \cdot 1$ (5)
Gastric juice (whole stomach) ..	$281 \cdot 4 \pm 33 \cdot 2$ (7)

The electrophoretic pattern of the gastric juice showed a main band that moved towards the anode and had the electrophoretic mobility of albumin. In addition there was a more rapidly moving anodal band (Band 6) and several bands of intermediate mobility. The latter are almost entirely mucopolysaccharides. The pattern resembles that found in human gastric juice (Piper, 1966; Piper *et al.*, 1963).

The response of the whole stomach (Group 1) and the isolated cardiogastric gland (Group 2) to histamine infusion was similar to other animals. After the start of the infusion the volume and acidity of gastric juice increased until after about one hour a peak was reached (Fig. 1). There was a tendency after the establishment of a peak for the secretion rate to diminish, while the acidity remained at a plateau. Supplementary subcutaneous injections of histamine caused a temporary increase in gastric output, but no increase in the concentration of acid. The injection of insulin was followed by a considerable increase in both the volume and the acidity of the gastric juice. The volume of gastric juice and the output of acid taken over all experiments were closely related $(r = 0 \cdot 80)$.

The pepsin concentration remained at a high and fluctuating level for some hours and then declined. The correlation of pepsin on volume was poor $(r = 0 \cdot 29)$, neither the injection of histamine nor of insulin affected the decline of peptic concentration. A typical result is shown in Fig. 2. The concentra-

tion of K^+ closely followed the concentration of pepsin in all the fluctuations ($r = 0.63$).

The concentration of all the ions measured in all experiments followed the usual relationships. Na^+ concentration fell as the acidity rose, and Cl^- and K^+ followed each other closely (Table 2).
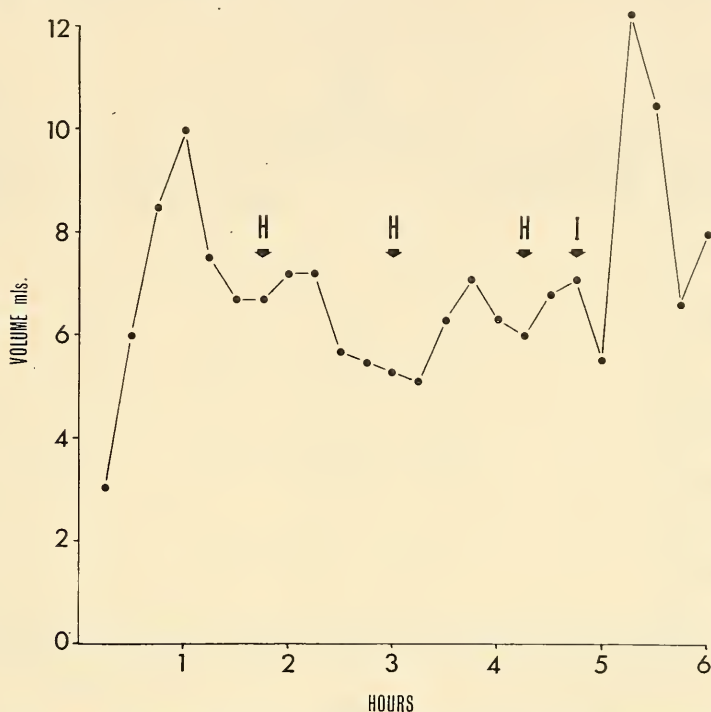

Fig. 1. Volume of gastric juice secreted from the whole stomach preparation described in the text. Histamine stimulation was commenced at 0 time. Note the rise to a peak after 1 hour and the decline despite continuing stimulation. Booster doses of histamine produced some increase in output, but a considerable increase in output followed the administration of insulin. H—histamine, I—insulin.

When comparing the maximum secretory capacity of the cardiogastric gland (Group 1) and the whole stomach including the cardiogastric gland (Group 2) it is necessary to take into account the effectiveness of the septum

TABLE 2

Correlation coefficients (r) for acidity (H) on Cl. Na, K and also for pepsin (P) on H, Na, Cl and K

The concentrations of all ions were in mEq/l. and pepsin in 10^3 units/l.

L.C. Ions			r	Pepsin (P)		r
H Vs Cl	..	..	0·78	P Vs H	..	−0·21
H Vs Na	..	...	−0·83	P Vs Cl	..	0·05
H Vs K	..	..	−0·27	P Vs Na	..	−0·05
				P Vs K	..	0·63

separating the gland from the body of the stomach. In one animal this septum was not adequate to exclude leakage from the body of the stomach into the cardiogastric gland area; the animal was excluded when the comparisons were made. Table 3 shows the maximum concentration of ions excreted by

four isolated cardiogastric gland areas and the maximum concentration from the whole stomach. There was no significant difference between any of the figures obtained for H^+, Cl^-, Na^+, K^+ and pepsin (Table 3).

The Hollander (1932) two-component theory of gastric secretion is based on the assumption that gastric juice is made up of two isotonic solutions.
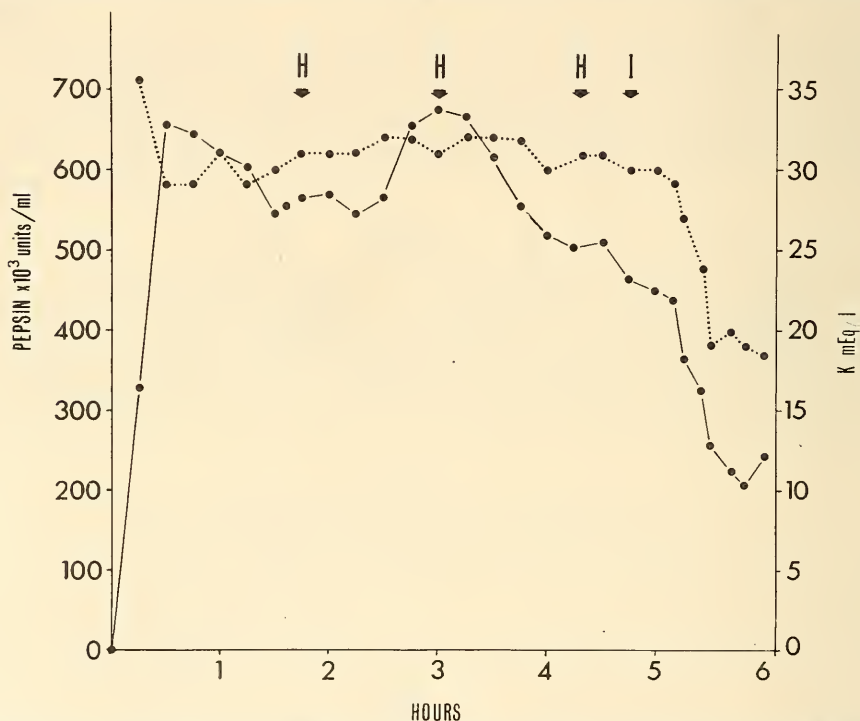

Fig. 2. Concentration of pepsin (Units 10^3/ml.) and K^+ (mEq/l.) from the gastric juice obtained from the animal shown in Fig. 1. The concentrations of pepsin and acid show similar fluctuations.

If this assumption is valid for the wombat there will be a linear relation between the concentrations x, y of different ions, taken two at a time, of the form—

$$y = a + bx \qquad (1)$$

where the constant b is given by

$$b = \frac{\sqrt{xy}}{\sqrt{xx}}$$

xy being the co-variance
xx being the variance.
The constant "a" is found by taking mean values in equation (1).

$$\bar{y} = a + b\bar{x}$$

or

$$a = \bar{y} - b\bar{x} \qquad (2)$$

The ionic relationships obtained in this manner are:

$$Cl^- = 0 \cdot 58H + 91 \cdot 4 \quad (r = 0 \cdot 77) \qquad (3)$$
$$Na^+ = -0 \cdot 521H + 86 \cdot 9 \quad (r = -0 \cdot 83) \qquad (4)$$
$$K^+ = -0 \cdot 083H + 26 \cdot 46 \quad (r = -0 \cdot 27) \qquad (5)$$

The nomenclature used to express the Hollander two-component theory is shown in Table 4.

If the two compounds are isotonic

$$p + q = b + c + d \qquad (6)$$

The relations between the ionic concentrations have been outlined by James (1957), and if x is the proportion of the parietal cell component in gastric juice, the relevant equations are:

$$Cl^- = bx + (c+d) \qquad (7)$$

$$Na^+ = (c+b)\,(1-x) \qquad (8)$$

$$K^+ = qx+d\,(1-x) \qquad (9)$$

$$H^+ = (p+b)\,x-b \qquad (10)$$

We require to know the concentrations of Cl^-, Na^- and K^+ in terms of H^+ as in equations (3), (4) and (5). Therefore by eliminating x from equations (7), (8), (9) and 10 we obtain:

$$Cl^- = \frac{b}{p+b}\,H + \frac{b^2}{p+b} + c + d \qquad (11)$$

$$Na^+ = -H\,\frac{(c+b)}{(p+b)} + p\,\frac{(c+b)}{(p+b)} \qquad (12)$$

$$K^+ = -H\,\frac{(d+q)}{(p+b)} + \frac{bq+pd}{p+b} \qquad (13)$$

$$H^+ = K\,\frac{c+d}{d-q} - q\,\frac{c+d}{d-q} \qquad (14)$$

The volumes of Cl^-, Na^+, K^+ and H^+ represent the total concentration of each ion in whole gastric juice. By solving the equations relevant for each ion, e.g., (11) and (3) for Cl^-, and (12) and (4) for Na^+ a series of values is obtained as in Table 5 which are in best agreement with the Hollander theory.

TABLE 3

Maximum concentration of Na, Cl, H and K at mEq/l. and of Pepsin $\times 10^3$ units/ml. $\pm$ S.D. obtained from four isolated cardiogastric glands and four total gastric pouch experiments

				Whole Stomach (Group 1)	Cardiogastric Gland (Group 2)
H	..	..	..	$129 \cdot 0 \pm \quad 4 \cdot 6$	$117 \cdot 0 \pm \quad 20 \cdot 1$
Cl		..	..	$170 \cdot 5 \pm \quad 2 \cdot 6$	$170 \cdot 5 \pm \quad 5 \cdot 0$
Na		..	..	$16 \cdot 3 \pm \quad 3 \cdot 8$	$24 \cdot 7 \pm \quad 10 \cdot 5$
K	..	..	..	$30 \cdot 0 \pm \quad 4 \cdot 8$	$19 \cdot 9 \pm \quad 4 \cdot 9$
P		..	..	$490 \cdot 7 \pm 137 \cdot 0$	$624 \cdot 7 \pm 232 \cdot 3$

In these results the parietal and non-parietal components are not isotonic.

The ionic relations calculated from equations (11) to (14), using the values in Table 5 are:

$$Cl^- = 0{\cdot}58H + 168 \qquad (15)$$

$$Na^+ = -0{\cdot}584H + 96 \qquad (16)$$

$$K^+ = -0{\cdot}0835H + 14 \qquad (17)$$

These relations are closest in the equations (3) to (5) in the present analysis. For comparison, we also found the best fit on the assumption that the total molarity of the non-parietal secretion was 170 mM., making it isotonic with the parietal secretion.

E

The best fit was found for the following values:

$$NaHCO_3 \quad b = 120 \text{ mM.}$$
$$NaCl \quad c = 35$$
$$KCl \quad d = 15$$
$$\overline{170}$$

which give the following theoretical relations:

$$Cl^- = 0 \cdot 42H + 100 \qquad (18)$$
$$Na^+ = -0 \cdot 54H + 90 \qquad (19)$$
$$K^+ = -0 \cdot 052H + 9 \qquad (20)$$

which again agree adequately with the experimental results (compare equations 3–5 with 18–20).

TABLE 4

Parietal cell secretion (Concentration)		Non-parietal cell secretion (Concentration)	
HCl	p	NaHCO$_3$	b
KCl	q	NaCl	c
		KCl	d

The figure of 170 mM. concentration assumed for both parietal and non-parietal solutions in the last analysis is not inconsistent with the measured osmolarity of 281·4 ml./l. (Table 1), since the latter figure is weighted strongly by the fully ionised solutes by a factor of two. However, the non-parietal figures given in Table 5 are probably inconsistent with the measured osmolarity.

TABLE 5

Parietal cell secretion (Concentration mEq/l.)			Non-parietal secretion (Concentration mEq/l.)		
HCl	p	167	NaHCO$_3$	b	232
KCl	q	0	NaCl	c	0
			KCl	d	33·4
Total concentration		167			265·4

The results were analysed to determine whether it was justified to group together the results for whole stomachs and for glands. From this analysis it appears that the Cl^-/H^+ and the Na^+/H^+ relations are the same for both gland and for stomach, but that the K^+/H^+ relations are different. We find for the cardiogastric gland alone

$$K = -0 \cdot 30H + 54 \quad (r = -0 \cdot 52)$$

whereas for the whole stomach alone

$$K = -0 \cdot 14H + 37 \cdot 2 \ (r = -0 \cdot 47).$$

The separation into two sets is seen to improve the correlation. These results taken together with the relations (3) and (4) give the Hollander analysis of the secretions (Table 6), which suggests that the parietal cells of the stomach secrete a small amount of HCl (20 mM.), whereas the parietal cells of the cardiogastric gland do not.

DISCUSSION

The wombat is a member of the Phascolomydiae family of the marsupials. The primitive forms of the marsupial separated from the ancestors of the present day mammals, in the remote past, probably about 70 million years ago.

Although in the intervening periods the marsupial has undergone considerable changes, especially in the larger Australian varieties, there is still an enormous gulf between the marsupial and the placental mammal. It is therefore of some interest to compare the secretory ability of this animal with that of more usual laboratory animals and man. On the whole the function of this "antique" stomach shows a remarkable resemblance to that of man. The stomach responded to histamine stimulation with a brisk ouptput of gastric juice and the concentration was only slightly lower than the maximum concentration achieved by man, dog and cat. The relationships of the different ions to one another were similar to those of the mammals. The Hollander two component theory still broadly fits the secretory pattern of this animal. The fit between theory and the observed results was not so close as has been observed in the cat (Milton *et al.*, 1963). This may be partly explained by the fewer number of experiments and the more restricted scatter of the observed ionic concentrations. The electrophoretic pattern of the gastric juice also resembled that of man.

TABLE 6

Parietal cell secretion				Non-parietal cell secretion			
		Gland	Stomach			Gland	Stomach
HCl ..	..	167	167	$NaHCO_3$	..	100	100
KCl ..	..	0	20	NaCl	..	35	35
				KCl ..	..	55	55

The close correlation between K^+ and pepsin, if confirmed in man, could be a useful indication of peptic concentration in human gastric function tests, as it is easier to measure K^+ concentration than pepsin.

Much has been written about the "maximal" gastric secretory capacity. These findings show that in this animal, when the stomach is secreting at close to the limit of its power as a response to histamine, then a considerable boost in secretion can be achieved by the addition of insulin to the stimulus. Stimulation with combined agents may therefore give a higher maximal level than the use of one agent alone.

The finding that the cardiogastric gland secretes juice of about the same concentration as the body of the stomach agrees with the histological findings that the cells composing the cardiogastric gland are similar to those of the corpus (Hingson and Milton, 1968). It was not feasible in these experiments to compare in detail the output of the cardiogastric gland to the rest of the stomach in terms of ml. per minute; a large number of animals would be required to do this and it would be necessary to standardise the size of the stomach and gland in each case. But in the intact animal the cardiogastric gland must secrete a large proportion of the gastric juice. The reason for the slight difference in the relationship between K^+/H^+ in the whole stomach and the gland is not clear, but it could be related to the very large number of chief cells in the gland area and their intimate relationship to the parietal cells, so that during acid secretion in the gland the cells extract some K^+ from the adjacent chief cells.

References

HARRISON, D. D., 1964.—Plasma pepsinogen as a test of gastric function. *Proc. Aust. Ass. clin. Biochem.*, 1(3): 75.

HINGSON, D. J., and MILTON, G. W., 1968.—The mucosa of the stomach of the wombat (*Vombatus hirsutus*) with special reference to the cardiogastric gland. PROC. LINN. SOC. N.S.W., 93: 69.

HOLLANDER, F., 1932.—Studies in gastric secretion. IV. Variations in the chlorine content of gastric juice and their significance. *J. biol. Chem.*, 97: 585.

HOME, E., 1808.—An account of some peculiarities in the anatomical structure of the wombat, with observations on the female organs of generation. *Phil. Trans. roy. Soc.*, 98: 304-312.

HOUSSAY, B. A., 1955.—"Human Physiology." McGrath, M., New York (2nd Ed.), p. 7.

JAMES, A. H., 1957.—"The Physiology of Gastric Digestion." The Williams and Wilkins Company, Baltimore, p. 50.

JOHNSTONE, J., 1898.—On the gastric glands of the marsupialia. *J. Linnean Soc.*, 27: 1-14.

LEHMANN, J., 1939.—Elektrometrische Mikrobestimmung von Chlor in Vollblut, Serum und Harn. *Acta Paediat.*, 26: 258-267.

MILTON, G .W., 1962.—The behaviour of gastric epithelium under various circumstances. *Ann. roy. Coll. Surg. Engl.*, 30: 351-367.

MILTON, G. W., SKYRING, A. P., and GEORGE, E. P., 1963.—Histamine secretory tests in cats. An evaluation of results based upon Hollander's two-component theory. *Gastroenterology*, 44(5): 642-653.

MULLER, E., 1942.—"Elektrometrische Masanalyse." Ed. 6, Leipzig.

MACKENZIE, W. C., 1918.—"The Gastrointestinal Tract in Monotremes and Marsupials." Critchley Parker, Melbourne.

NASSET, E. S., 1953.—Gastric secretion in the beaver (*Castor canadensis*). *J. Mammology*, 34: 204-209.

PIPER, D. W., 1966.—Personal communication.

PIPER, D. W., STIEL, M. C., and BUILDER, J. E., 1963.—The electrophoretic pattern of normal human gastric juice and of the gastric juice of patients with gastric ulcer and gastric cancer. *Gut*, 4: 236-242.

SMITH, J. R., FREYTAG, F. C., HUTZINGA, H., and CLARK, L. P., 1911.—Preliminary studies on the structure and function of the cardiogastric gland of the beaver. *Colorado-Wyoming Acad. Sci. J.*, 3: 58.

SPECTOR, W. S., 1956.—"Handbook of Biological Data." Editor Spector, W. B., Saunders, Philadelphia.